

Claims

1 1. A nucleotide sequence comprising a coding sequence for a mutant
2 purine cleaving enzyme having different biological activity than a wild type
3 purine cleaving enzyme.

1 2. The nucleotide sequence of claim 1 wherein the different biological
2 activity is greater biological activity.

1 3. The nucleotide sequence of claim 1 wherein the mutant purine
2 cleaving enzyme is a nucleoside hydrolase.

1 4. The nucleotide sequence of claim 1 wherein the mutant purine
2 cleaving enzyme is a purine nucleoside phosphorylase.

1 5. The nucleotide sequence of claim 3 wherein the mutant purine
2 cleaving enzyme is an *E. coli* nucleoside hydrolase.

1 6. The nucleotide sequence of claim 4 wherein the mutant purine
2 cleaving enzyme is an *E. coli* purine nucleoside phosphorylase.

1 7. The nucleotide sequence of claim 1 wherein the coding sequence
2 encodes an enzyme having different biological activity cleaving a nucleoside
3 analog than a wild-type enzyme.

1 8. The nucleotide sequence of claim 1 wherein said enzyme is
2 selected from the group consisting of: *Leishmania donovani* nucleoside hydrolase;
3 *Trichomonas vaginalis* purine nucleoside phosphorylase; *Trypanosoma cruzi*
4 nucleoside hydrolase; *Schistosoma mansoni* purine nucleoside phosphorylase;
5 *Leishmania tropica* nucleoside hydrolase; *Crithidia fasciculata* nucleoside
6 hydrolase; *Aspergillus* and *Penicillium* nucleoside hydrolase; *Erwinia carotovora*
7 purine nucleoside phosphorylase; *Helix pomatia* purine nucleoside phosphorylase;
8 *Ophiodon elongatus* (lingcod) purine nucleoside phosphorylase; *Salmonella*
9 *typhimurium* purine nucleoside phosphorylase; *Bacillus subtilis* purine nucleoside
10 phosphorylase; *Clostridium* purine nucleoside phosphorylase; mycoplasma purine
11 nucleoside phosphorylase; *Trypanosoma gambiense* nucleoside hydrolase;
12 *Trypanosoma brucei* purine nucleoside phosphorylase (methylthioadenosine
13 phosphorylase); 5'-methylthioadenosine phosphorylase from *Sulfolobus*
14 *solfataricus* and 5'-methylthioadenosine/S-adenosyl homocysteine nucleosidase
15 from *E. coli*.

1 9. The nucleotide sequence of claim 1 wherein said coding sequence
2 is for a mutant *E. coli* purine nucleoside phosphorylase containing a complete
3 open reading frame and encodes an amino acid sequence depicted as residues 1-
4 239 of SEQ ID NO: 2.

1 10. The nucleotide sequence of claim 1 wherein said nucleotide
2 sequence comprises residues 1-720 of SEQ ID NO: 1.

1 11. The nucleotide sequence of claim 1 wherein said coding sequence
2 is for a mutant *E. coli* purine nucleoside phosphorylase containing a complete
3 open reading frame and encodes an amino acid sequence depicted as residues 1-
4 239 of SEQ ID NO: 4.

1 12. The nucleotide sequence of claim 1 wherein said nucleotide
2 sequence comprises residues 1-720 of SEQ ID NO: 3.

1 13. The nucleotide sequence of claim 1 wherein said coding sequence
2 is for a mutant *E. coli* purine nucleoside phosphorylase containing a complete
3 open reading frame and encodes an amino acid sequence of mutant selected from
4 the group consisting of: M65A, M65I, M65Q, H5N, A157F, A157L, E180D,
5 E180N, E180S, E180T, M181A, M181L, M181N, M181V, M181E, E182A,
6 E182Q, E182V, D205A and D205N.

1 14. A vector comprising the nucleotide sequence of claim 1.

1 15. The vector of claim 14 wherein the nucleotide sequence encodes a
2 mutant *E. coli* purine nucleoside phosphorylase protein, said nucleotide sequence
3 depicted as residues 1-720 of SEQ ID NO: 1.

1 16. The vector of claim 14 wherein the nucleotide sequence encodes a
2 mutant *E. coli* purine nucleoside phosphorylase protein depicted as residues 1-239
3 of SEQ ID NO: 2.

1 17. The vector of claim 14 wherein the nucleotide sequence encodes a
2 mutant *E. coli* purine nucleoside phosphorylase protein, said nucleotide sequence
3 depicted as residues 1-720 of SEQ ID NO: 3.

1 18. The vector of claim 14 wherein the nucleotide sequence encodes a
2 mutant *E. coli* purine nucleoside phosphorylase protein depicted as residues 1-239
3 of SEQ ID NO: 4.

1 19. The vector of claim 14 wherein said vector further comprises at
2 least a portion of one component to aid delivery to target cells selected from the
3 group consisting of: a virus, bacteria, mammalian cell, non-mammalian cell,
4 DNA molecule, and modified DNA molecule.

1 20. The vector of claim 14 selected from the group consisting of: a
2 retroviral vector, an adenoviral vector, an adeno-associated viral vector, a herpes
3 vector, a viral vector and a plasmid.

1 21. The vector of claim 14 wherein the nucleotide sequence encodes a
2 mutant *E. coli* purine nucleoside phosphorylase protein selected from the group

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3 consisting of: M65A, M65I, M65Q, H5N, A157F, A157L, E180D, E180N,
4 E180S, E180T, M181A, M181L, M181N, M181V, M181E, E182A, E182Q,
5 E182V, D205A and D205N.

1 22. A host cell transformed with a vector comprising a nucleotide
2 sequence encoding a mutant purine cleaving enzyme having different biological
3 activity than a wild-type purine cleaving enzyme.

1 23. The host cell of claim 22 wherein the vector comprises the
2 nucleotide sequence depicted as residues 1-720 of SEQ ID NO: 1.

1 24. The host cell of claim 22 wherein the nucleotide sequence encodes
2 a mutant *E. coli* purine nucleoside phosphorylase protein depicted as residues 1-
3 239 of SEQ ID NO: 2.

1 25. The host cell of claim 22 wherein the vector comprises the
2 nucleotide sequence depicted as residues 1-720 of SEQ ID NO: 3.

1 26. The host cell of claim 22 wherein the nucleotide sequence encodes
2 a mutant *E. coli* purine nucleoside phosphorylase protein depicted as residues 1-
3 239 of SEQ ID NO: 4.

1 27. A recombinant virus which is capable of transferring a gene to a
2 target cell and which comprises the nucleotide sequence of claim 1.

1 28. The virus of claim 27 wherein the nucleotide sequence is depicted
2 as residues 1-720 of SEQ ID NO: 1.

1 29. The virus of claim 27 wherein the nucleotide sequence encodes a
2 mutant *E. coli* purine nucleoside phosphorylase protein depicted as residues 1-239
3 of SEQ ID NO: 2.

1 30. The virus of claim 27 wherein the nucleotide sequence is depicted
2 as residues 1-720 of SEQ ID NO: 3.

1 31. The virus of claim 27 wherein the nucleotide sequence encodes a
2 mutant *E. coli* purine nucleoside phosphorylase protein depicted as residues 1-239
3 of SEQ ID NO: 4.

1 32. The virus of claim 27 wherein the nucleotide sequence encodes a
2 mutant *E. coli* purine nucleoside phosphorylase protein selected from the group
3 consisting of: M65A, M65I, M65Q, H5N, A157F, A157L, E180D, E180N,
4 E180S, E180T, M181A, M181L, M181N, M181V, M181E, E182A, E182Q,
5 E182V, D205A and D205N.

1 33. A host cell transformed with the virus of claim 27.

1 34. A process for impairing a cell comprising the steps of:

2 (a) introducing the nucleotide sequence of claim 1 into a target cell;

3 and

4 (b) contacting said target cell with an effective amount of a prodrug.

1 35. The process for impairing a cell of claim 34 wherein the nucleotide
2 sequence encodes a mutant *E. coli* purine nucleoside phosphorylase protein, said
3 nucleotide sequence depicted as residues 1-720 of SEQ ID NO: 1.

1 36. The process for impairing a cell of claim 34 wherein the nucleotide
2 sequence is for a mutant *E. coli* purine nucleoside phosphorylase containing a
3 complete open reading frame and encodes an amino acid sequence depicted as
4 residues 1-239 of SEQ ID NO: 2.

1 37. The process for impairing a cell of claim 34 wherein the nucleotide
2 sequence encodes a mutant *E. coli* purine nucleoside phosphorylase protein, said
3 nucleotide sequence depicted as residues 1-720 of SEQ ID NO: 3.

1 38. The process for impairing a cell of claim 34 wherein the nucleotide
2 sequence is for a mutant *E. coli* purine nucleoside phosphorylase containing a

3 complete open reading frame and encodes an amino acid sequence depicted as
4 residues 1-239 of SEQ ID NO: 4.

1 39. The process for impairing a cell of claim 34 wherein the nucleotide
2 sequence is that encoding a mutant *E. coli* purine nucleoside phosphorylase
3 protein selected from the group consisting of: M65A, M65I, M65Q, H5N, A157F,
4 A157L, E180D, E180N, E180S, E180T, M181A, M181L, M181N, M181V,
5 M181E, E182A, E182Q, E182V, D205A and D205N.

1 40. The process for impairing a cell of claim 34 wherein said target cell
2 is selected from the group consisting of: tumor cells and pathogenic virus-infected
3 cells, and abnormally proliferating cells.

1 41. The process for impairing a cell of claim 34 wherein said prodrug
2 is a purine nucleoside analog.

1 42. The process for impairing a cell of claim 34 wherein said prodrug
2 is selected from the group consisting of: 9-(2-deoxy- β -D-ribofuranosyl)-6-
3 methylpurine, 9-(β -D-ribofuranosyl)-2-amino-6-chloro-1-deazapurine, 7-(β -D-
4 ribofuranosyl)-3-deazaguanine, 9-(β -D-arabinofuranosyl)-2-fluoroadenine, 2-
5 fluoro-2'-deoxyadenosine, 2-fluoro-5'-deoxyadenosine, 2-chloro-2'-
6 deoxyadenosine, 5'-amino-5'-deoxyadenosine, 9-(α -D-ribofuranosyl)-2-
7 fluoroadenine, 9-(2,3-dideoxy- β -D-ribofuranosyl)-6-methylpurine, 9-(2,3-

8 dideoxy- β -D-ribofuranosyl)-2-fluoroadenine, 9-(3-deoxy- β -D-ribofuranosyl)-2-
9 fluoroadenine, 9-(3-deoxy- β -D-ribofuranosyl)-6-methylpurine, 9-(6-deoxy- β -D-
10 allofuranosyl)-6-methylpurine, 9-(6-deoxy- β -D-allofuranosyl)-2-fluoroadenine, 9-
11 (α -L-lyxofuranosyl)-2-fluoroadenine, 9-(α -L-lyxofuranosyl)-6-methylpurine, 9-
12 (6-deoxy- α -L-talofuranosyl)-6-methylpurine, 9-(6-deoxy- α -L-talofuranosyl)-2-
13 fluoroadenine, 5'-amino-5'-deoxyadenosine, 9-(6,7-dideoxy- β -D-hept-6-
14 ynofuranosyl)-6-methylpurine, 9-(6,7-dideoxy- α -L-hept-6-ynofuranosyl)-6-
15 methylpurine, 9-(2,3-dideoxy-3-hydroxymethyl- α -D-ribofuranosyl)-6-
16 thioguanine.

1 43. The process for impairing a cell of claim 34 wherein said step of
2 introducing the nucleotide sequence of claim 1 into a target cell is by
3 administering a virus comprising said sequence to the target cell.

1 44. The process for impairing a cell of claim 34 wherein said step of
2 introducing the nucleotide sequence of claim 1 into a target cell by a method
3 selected from the group consisting of lipofection, biolistic administration and
4 direct injection of DNA.

1 45. A process for impairing a cell comprising the steps of:

- 2 (a) administering a mutant PNP enzyme to a target; and
3 (b) delivering an effective amount of a prodrug.

1 46. The process for impairing a cell of claim 45 wherein the target is
2 selected from the group consisting of: a virus, a bacterium, a protozoan, a cell, a
3 tissue, an organ, a tumor and a combination thereof.

1 47. A process for impairing a cell comprising the steps of:
2 (a) administering to a target PNP mutant enzyme M65V; and
3 (b) delivering a prodrug such that an effective amount of the prodrug is
4 cleaved by the enzyme.

1 48. A process for impairing a cell comprising the steps of:
2 (b) administering to a target PNP mutant enzyme M65V; and
3 (b) delivering a prodrug selected from the group consisting of: 9-(6,7-
4 dideoxy- α -L-hept-6-ynofuranosyl)-6-methylpurine; 9-(α -L-lyxofuranosyl)-2-F-
5 adenine; and 9-(6-deoxy- α -L-talofuranosyl)-6-methylpurine.

1 49. A purified mutant purine cleaving enzyme having different
2 biological activity than a wild type purine cleaving enzyme.

1 50. The enzyme of claim 49 wherein the enzyme is a mutant *E. coli*
2 purine nucleoside phosphorylase.

1 51. The enzyme of claim 50 wherein the mutant *E. coli* purine
2 nucleoside phosphorylase is M65V.

1 52. The enzyme of claim 50 wherein the mutant *E. coli* purine
2 nucleoside phosphorylase is A157V.

1 53. The enzyme of claim 50 wherein the mutant *E. coli* purine
2 nucleoside phosphorylase is selected from the group consisting of: M65A, M65I,
3 M65Q, H5N, A157F, A157L, E180D, E180N, E180S, E180T, M181A, M181L,
4 M181N, M181V, M181E, E182A, E182Q, E182V, D205A and D205N.

1 54. A commercial kit for impairing a cell comprising:
2 a vector containing a nucleotide sequence encoding an amino acid
3 sequence depicted as residues 1-239 of SEQ ID NO: 2; and
4 instructions for use.

1 55. A commercial kit for impairing a cell comprising:
2 a purified mutant purine nucleoside phosphorylase protein depicted as
3 residues 1-239 of SEQ ID NO: 2; and
4 instructions for use.

1 56. A commercial kit for impairing a cell comprising:
2 a recombinant virus containing a nucleotide sequence encoding a mutant
3 *E. coli* purine nucleoside phosphorylase protein depicted as residues 1-239 of SEQ
4 ID NO: 2; and instructions for use.